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Lobsters, Lymph and Histamine-Liberation

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Introduction

In 1891, *Heidenhain* described experiments on the formation of lymph in which he characterised lymphagogues of the first and second orders; the first caused a secretion of a concentrated lymph, and included materials such as decoctions of crustacean muscle; the second promoted a flow of dilute lymph, and included saline solutions. He took the view that the lymphagogues of the first order stimulated endothelial cells to active "secretion" of lymph. *Starling*, in 1894, took up the subject in the light of his studies on formation of tissue fluid. He rejected the conception of active secretion, and produced convincing evidence that first order lymphagogues acted simply by damaging capillary permeability and by increasing capillary flow. These materials were then left as capillary poisons of an unspecified character.

Heidenhain's observations are, however, suggestive in another direction. He describes how he came to test crustacean muscle as follows:

"Ich hatte um die Zeit meiner Beschäftigung mit diesen Dingen zufällig Gelegenheit, den von Pathologen oft beschriebenen Ausbruch von Nesselquaddeln nach Krebsgenuss selbst zu beobachten. Fräulein A. B. bekam bald, nachdem sie einige Krebse gegessen, nicht bloss Urticaria in gewöhnlicher Form, sondern dabei ein starkes diffuses Hautödem in der linken Halsgegend, welches vom Unterkiefer bis fast zum Schlüsselbein reichte und mehrere Stunden stehen blieb. Diese Beobachtung gab Anlass zu folgendem Versuche".

He then found that extracts of crayfish and lobster muscle were effective lymphagogues; but he also made some other observations, of which his description of the infusion of one of his decoctions into a dog is specially significant. "Unmittelbar nach der Injektion suchte das Tier mit der Schnauze fortwährend auf der Haut herum, wie es stark mit Flöhen besetzte Hunde zu tun pflegen. Es schienen also juckende Empfindungen vorhanden zu sein." If these remarks are taken together with *Starling*'s observation that first order lymphagogues cause haemoconcentration, a fall in arterial blood pressure, a rise in portal venous pressure, and swelling of a limb, there is a substantial *prima facie* case for suspecting a release of histamine in the action of these substances. That histamine, itself, could be the active principle was improbable, since the muscles were first extracted with alcohol before preparing the decoction.

The experiments in this paper were designed initially to test this suspicion that extracts of crustacean muscle owed their lymphagogue action to histamine release; and also to test the corollary that the recently described histamine liberators (see [6] for a review) should also promote a flow of lymph.

Methods

Histamine-release. To test for histamine release, a tissue with low spontaneous release but as sensitive as possible to histamine liberators was required; there was no expectation that the decoctions described by *Heidenhain* would be very potent in releasing histamine since both he and *Starling* required fairly large volumes (of the order of 20–50 ml.) to produce their effects. Accordingly the cat's perfused skin flap preparation was employed (*Feldberg* and *Paton*, 1951) since it is probably the most sensitive specific test available, and has the advantage of a low spontaneous histamine release. The flap was prepared in the usual way from cats anaesthetised with chloralose.

Assay of histamine. Histamine in the perfusate from the cat's skin was assayed on the guineapig ileum. In a few experiments, low doses of mepyramine were added to the test samples to verify that the material released had the properties of histamine.

Lymphagogue actions. The thoracic duct was cannulated, after heparinization (10–20 mg./kg.), in cats anaesthetised with chloralose (80 mg./kg.). The animals also had tracheal cannulae so that artificial respiration could be administered when required. Records of blood pressure were taken by means of a heparinised siliconed cannula in the carotid artery. The flow of lymph from the duct was recorded by a drop counter which made a mark on a smoke drum with each drop; there were about 30 drops to 1 cc. of lymph.

Preparation of lymphagogue decoctions. Heidenhain's decoctions were prepared as follows: The flesh of edible mussels (Mytilus Edulis), the common lobster (Homarus) or the rock lobster (Palinurus vulgaris) was dissected out from freshly scalded specimens and ground up in a large volume of 95% alcohol (spirits of wine). The grindings were washed with the alcohol until the filtrate through a *Buchner* funnel was colourless. The alcohol-washed flesh was then dried and stored in vacuo. 100 gm. of flesh yielded about 20 gm. dried

powder. For preparing the decoction, 5 gm. of the powder was boiled in 100 ml. of saline for one or two hours, and the filtrate used; it did not contain histamine.

Results

Histamine-release by lymphagogues. The first experiments were to test whether the lymphagogues could release histamine. Fig. 1 shows the responses to injection of 1 cc. of these three decoctions into the arterial cannula of the perfused cat's skin preparation.

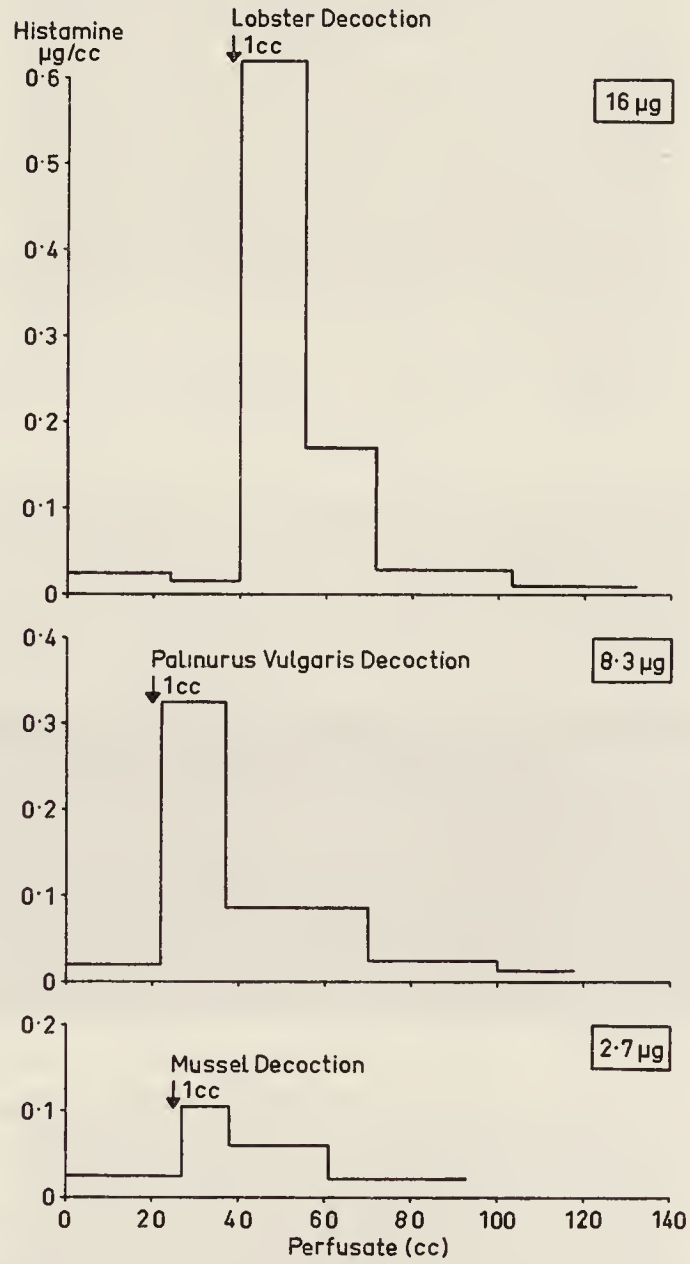

Fig. 1. Cat's isolated perfused skin preparations. Outputs of histamine in response to lobster, palinurus vulgaris and mussel decoction.

In each case, an unequivocal release of histamine occurred. The amounts released are not large, but the dose of decoction used was only a small fraction of the amounts used by *Heidenhain* and *Starling*.

In addition, some simple tests were made by intradermal injection into a human skin. The flexor surface of the forearm was used; 0.03 ml. of each decoction was injected directly intradermally. Each decoction produced a distinct wheal and flare and a sensation

of itching, roughly comparable to that produced by Compound 48/80, 1 μg./ml. It was noticed incidentally, that when a second decoction of mussel was tested three weeks after an earlier intradermal test of a mussel extract, the site of the earlier test showed a delayed erythematous itching reaction. This indicates that as well as any direct histamine releasing action by the material, local sensitization can also take place.

The Action of Histamine Liberators on the Flow of Lymph

Figs. 2, 3, 4, 5, and 6 show the lymphagogue action of histamine, and of the histamine liberators, propamidine, d-tubocurarine, morphine and Compound 48/80. Sometimes the acceleration of flow of lymph was striking, increasing temporarily to ten times the normal rate, or more. Occasionally, however, only modest responses were seen, sometimes because the blood pressure of the animal was low or because previous doses of liberator had been given, but sometimes without obvious cause. Whenever a very effective lymphagogue action had been obtained any subsequent dose of a histamine liberator was less effective. Sometimes, the increased flow came in in two

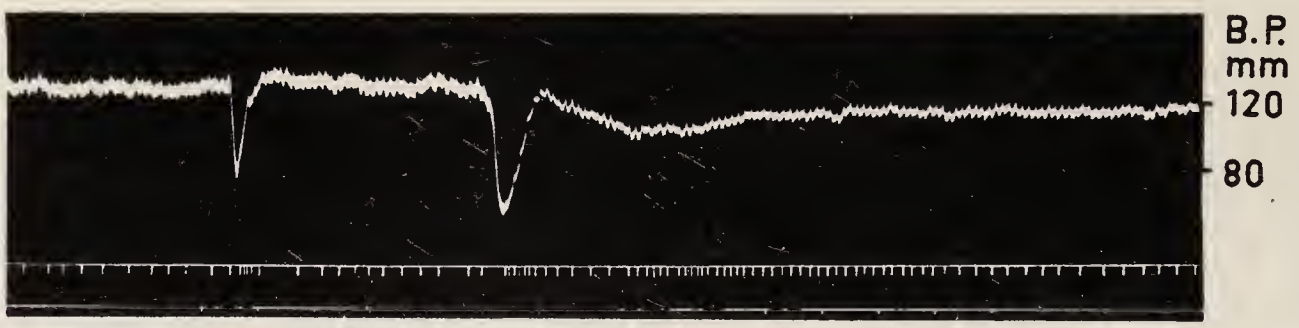

Fig. 2. Cat, chloralose, Record of blood pressure, lymph flow, and time (minutes). Intravenous injection first of histamine (10 μg.), then propamidine (3 mg.).

phases; an initial burst, leading to a temporary reduction in flow, after which the second sustained phase followed (figs 2 and 4). This pattern of response was obviously paralleled by characteristic blood pressure changes. The initial delayed depressor response is followed by a temporary rise in pressure and tachycardia due to suprarenal medullary secretion; finally, a more prolonged depression of blood pressure ensues.

It was hoped initially that it might be possible to demonstrate the appearance of histamine in the lymph as a result of the action of histamine-liberators and to relate this to the lymph flow. However, no histamine at all could ever be found in the lymph, because of its

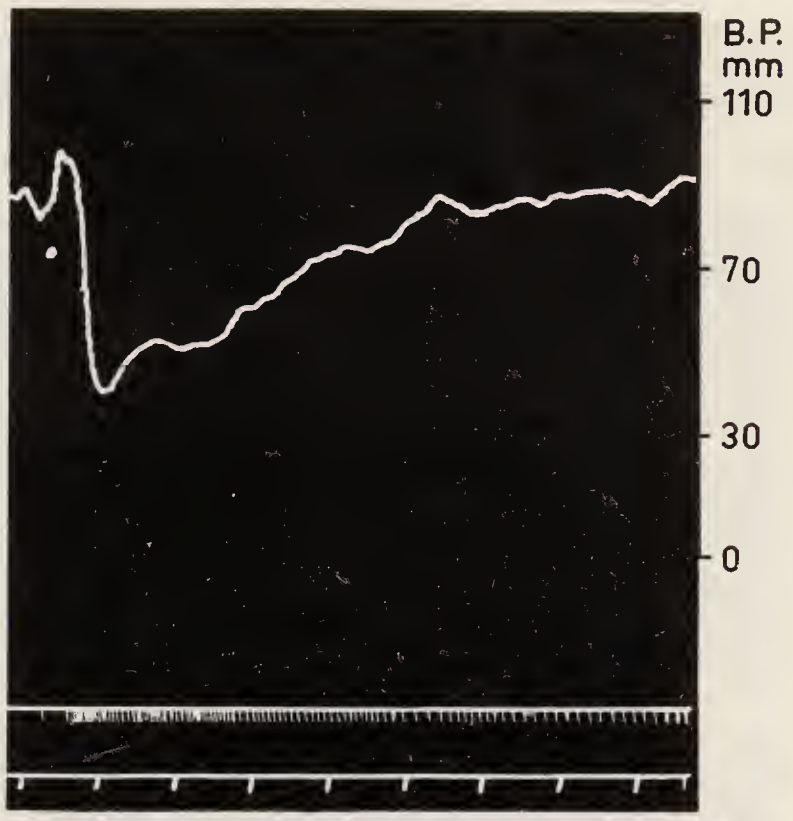

Fig. 3. Cat, chloralose. Record of blood pressure, lymph flow and time (minutes). Response to propamidine, 5 mg.; intravenous injection

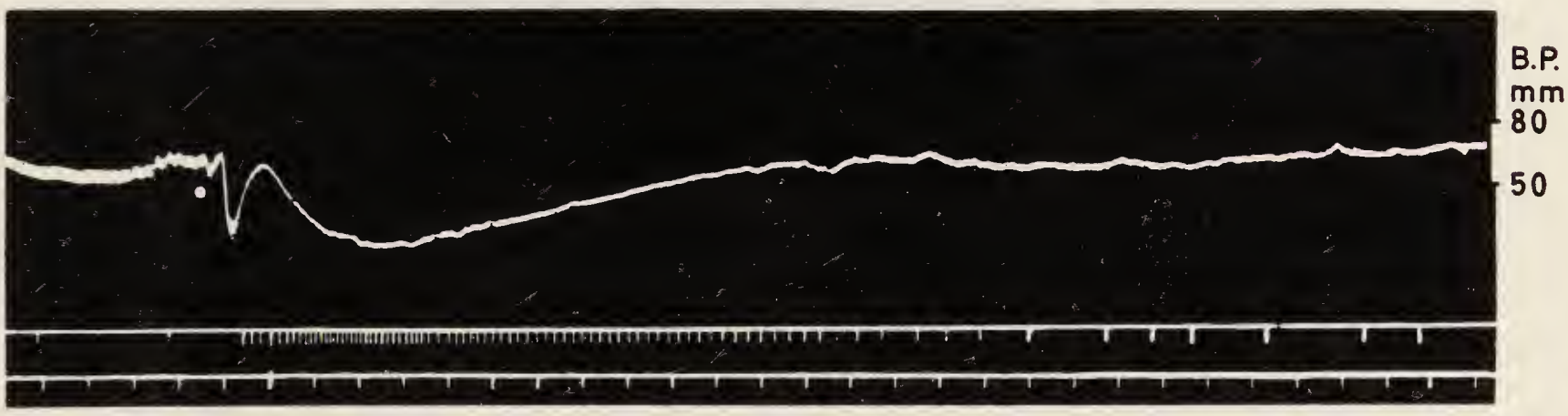

Fig. 4. Cat, chloralose. Record of blood pressure, lymph flow, and time (minutes). Response to 2 mg./kg. d-tubocurarine; intravenous injection

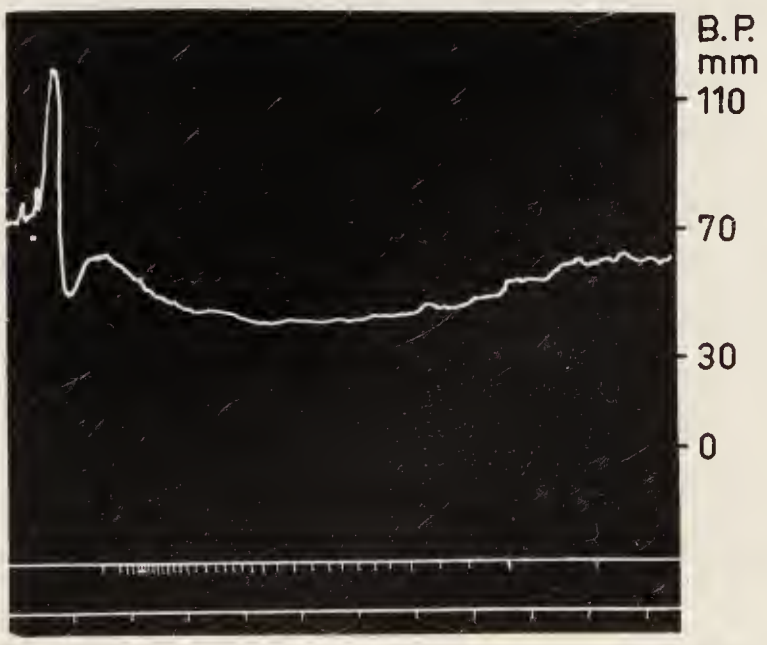

Fig. 5. Cat, chloralose. Record of blood pressure, lymph flow and time (minutes). Response to morphine 10 mg.; intravenous injection

remarkable capacity to destroy histamine. In one sample as much as 80 μg. of histamine/hr./ml. lymph was destroyed at room temperature. In two experiments, tests were made as to whether lymphagogues caused any change in the output of histaminase in the lymph. But no consistent change was found, apart from the increase in total output associated with increased lymph flow.

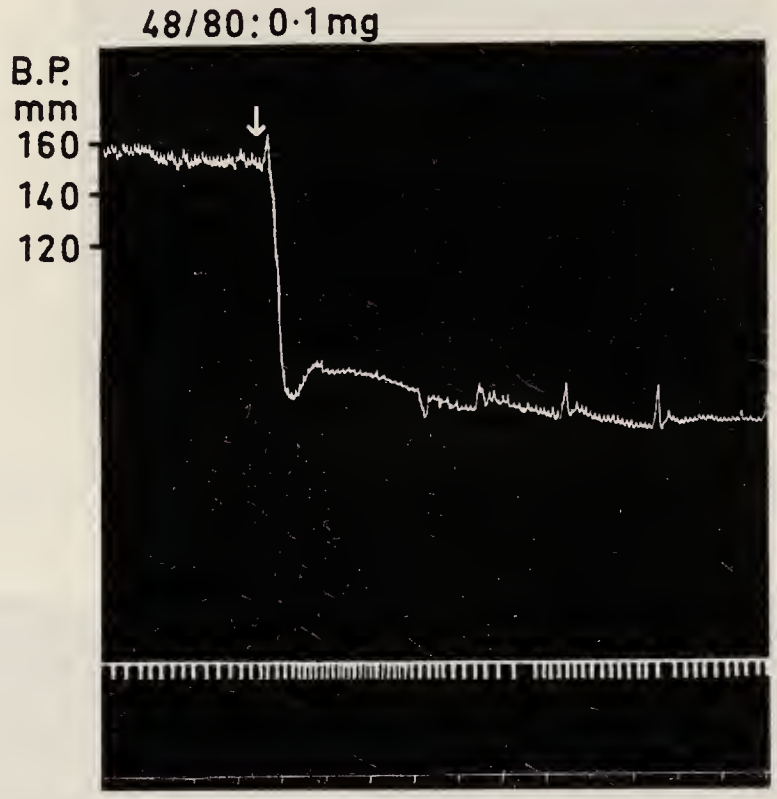

Fig. 6. Cat, chloralose. Record of blood pressure, lymph flow, and time (minutes). Response to 100 μg. Compound 48/80; intravenous injection

Experiments with Other Substances

The results just described showed that histamine-liberation and lymphagogue action are closely associated, and allow one to conclude that it is histamine released from the tissues which causes the enhanced lymph flow. The opportunity was taken, however, to examine the actions on lymph flow of other substances capable of altering the blood pressure.

The action of histamine has already been described. It was found, as would be expected, that mepyramine (1 mg.) given intravenously before an injection of 20 μg. histamine could almost abolish its lymphagogue effect at the same time as attenuating the depressor response. Acetylcholine, in doses producing a similar fall in blood pressure to that produced by histamine, was less effective, sometimes producing a few extra drops of lymph, sometimes reducing the flow a little. Hexamethonium, in a dose of 1–4 mg./kg., which produced a sustained fall in blood pressure, also had little significant action,

although there was a suggestion of a slight acceleration of flow in the first few minutes followed by a slight reduction for a longer period.

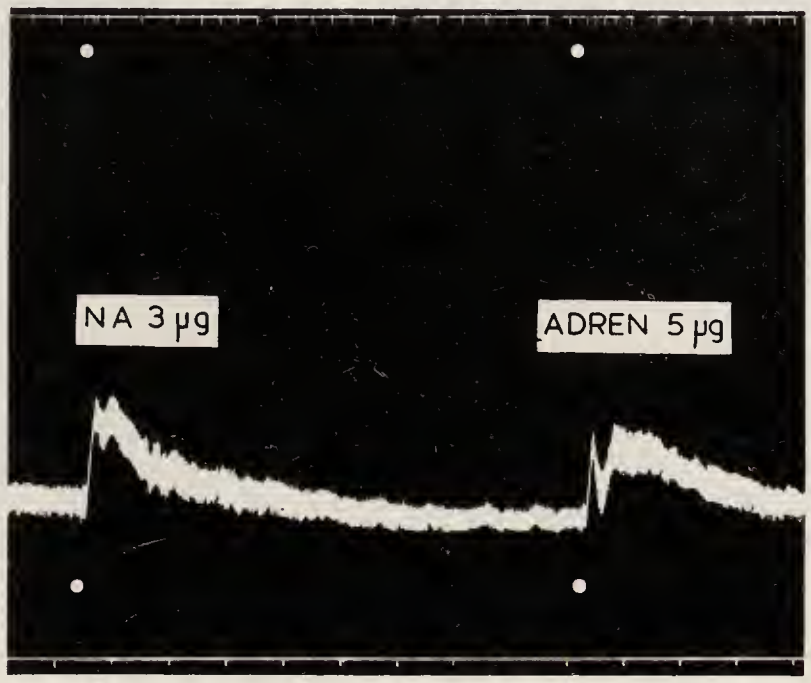

Fig. 7. Cat, chloralose. Record of lymph flow, blood pressure, and time (minutes). Response to 3 μg. noradrenaline and 5 μg. adrenaline, intravenous injection

Sympathomimetic Amines

With adrenaline and noradrenaline, however, more pronounced effects were seen. With moderate doses (fig. 7), a brief spurt of lymph followed the injection, and flow was then somewhat reduced thereafter. With a large dose of adrenaline (fig. 8), the initial spurt led to a cessation of flow for some minutes, after which a distinctly augmented production of lymph occurred. The net effect of administering such a dose, despite the cessation of flow, was to increase the production from the anticipated 20 drops in 7 min. to about 40 drops. The acceleration of flow occurred while the blood pressure was still raised. This lymphagogue response could be produced repeatedly without significant attenuation. Noradrenaline produced the same sequence of events. Tyramine and β-phenylethylamine (fig. 9) did the same, although the period of cessation of flow was somewhat more prolonged. In every case, the secondary increase in flow more than compensated for the reduction seen during the peak of the pressor response.

Histamine Release by Sympathomimetic Amines

The possibility that adrenaline may release histamine has been canvassed not infrequently (v. 6). Histamine release from lung does

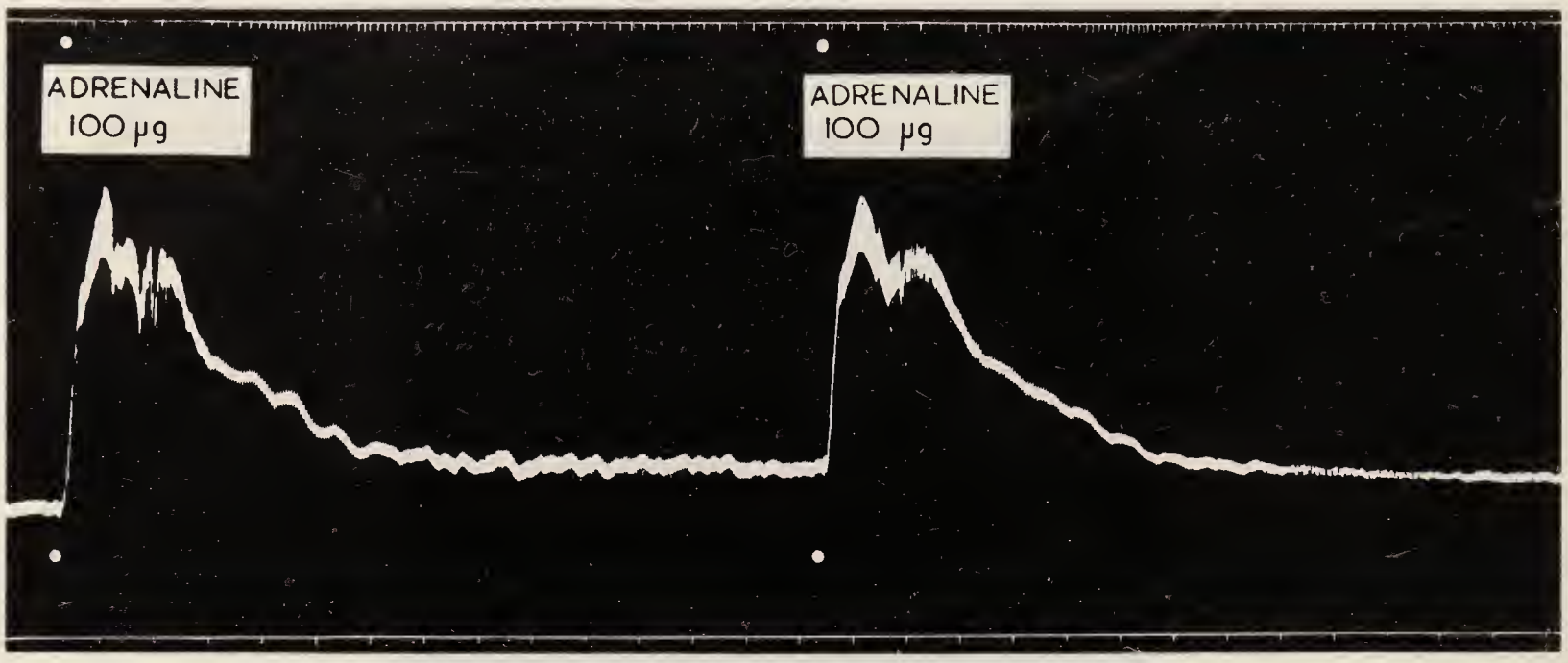

Fig. 8. Cat, chloralose. Record of lymph flow, blood pressure, and time (minutes). Response to 100 μg. adrenaline, twice; intravenous injection

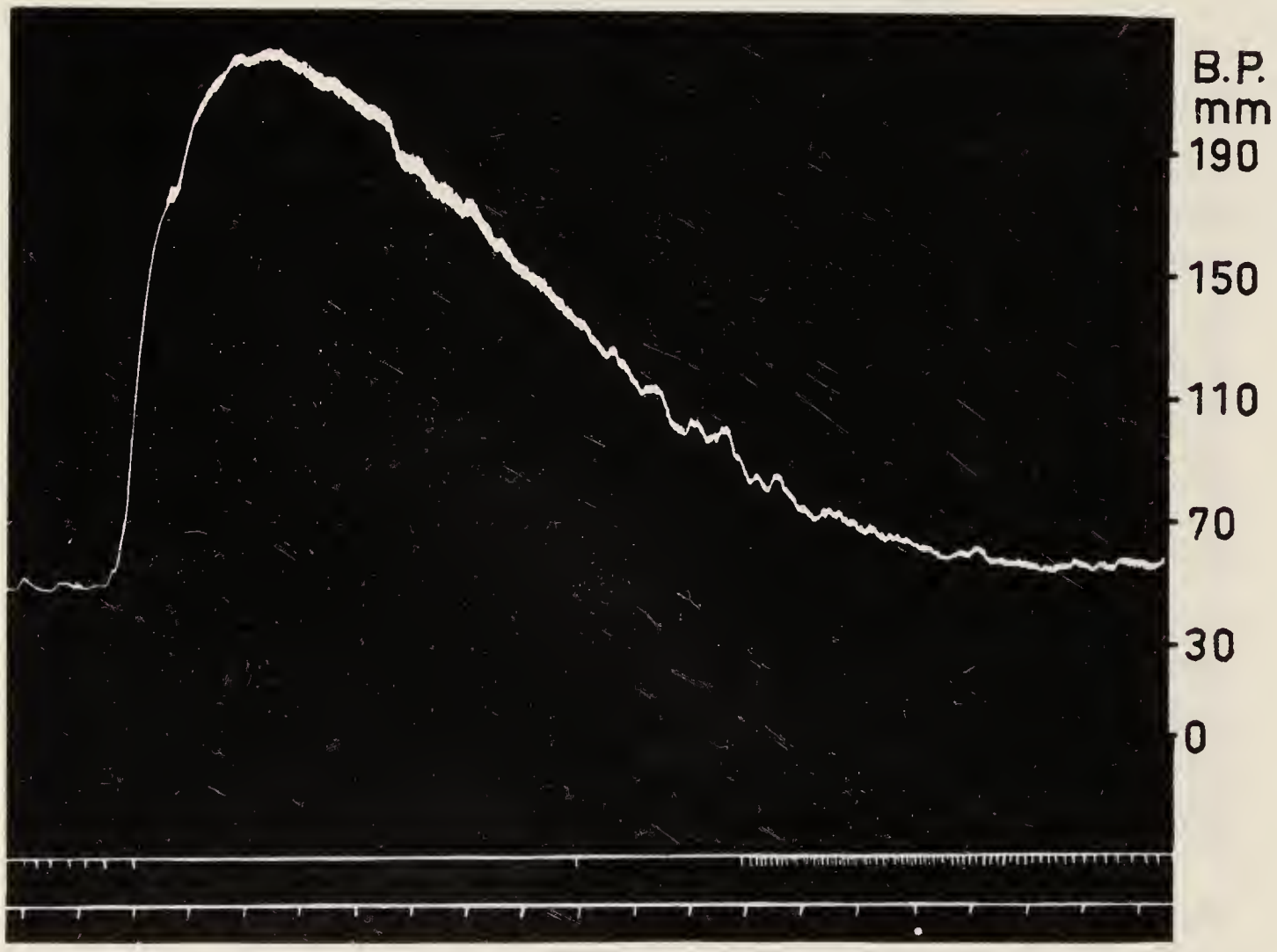

Fig. 9. Cat, chloralose. Record of blood pressure, lymph flow, and time (minutes). Response to β-phenylethylamine 10 mg.; intravenous injection

not seem to be significant (*Dale* and *Richards*, 1927). Some evidence pointing to an increase in the histamine content of whole blood in man has been advanced; but it is difficult to infer from this whether plasma histamine was raised, or a redistribution of histamine-containing cells in the blood took place. *Mongar* and *Whelan* (1953) failed to obtain evidence for such release following infusion of adrenaline into the human forearm. A suggestive early observation was that by *Bainbridge* and *Trevan* (1917), who found that adrenaline intraportally in the dog produced a shock-like state with engorgement of the liver and a rise of portal venous pressure, together with an increase in thoracic lymph flow.

It seemed worth while to test this point further, and two exploratory experiments were made. In the first, *Bainbridge* and *Trevan*'s experiment was repeated. In a dog under chloralose-urethane anaesthesia, in which thoracic lymph flow and blood pressure were recorded, adrenaline 1 mg. was injected intraportally. This led to a three or four-fold increase in lymph flow (0.12 cc./min. to 0.55 cc./min., and 0.11 cc./min. to 0.36 cc./min. in a second test), and a vigorous rise in blood pressure for 1–2 minutes followed by a fall, normal pressure being restored in 5–6 minutes. No rise in plasma histamine could be detected, and there were no signs of peripheral circulatory shock. The only suggestive observation was that the lymph became less coagulable. Before the adrenaline injection, it clotted in less than 30 minutes. Three minutes after the injection clotting took 1 hr. 40 min., and a sample 9 minutes later was unclotted in 3½ hours. Later samples clotted in 60 min. and 50 min. The blood coagulation time (which can be distinctly prolonged by peptone and other histamine-liberators) was hardly affected, although at the peak of the effect on the lymph coagulation time, blood clotted in 11 minutes compared to the normal 3–4 minutes. These results, however, could be attributed as readily to the effect of ischemia on the dog liver and its mast cells as to specific histamine release.

It seemed, therefore, that adrenaline had, at most, a very limited ability to mobilize histamine or (in the dog) heparin, and that a more sensitive test was required. Accordingly, on the perfused skin preparation, two experiments were made, with 100 μg. adrenaline and 1 mg. noradrenaline respectively given into the arterial perfusion cannula. Both injections produced very severe vasoconstriction. The effluent was assayed for histamine against standard hista-

mine solutions containing adrenaline or noradrenaline in the dilution expected if the injected quantities were collected in the effluent. No increase (< 0.02 μg./ml.) in histamine content of the effluent could be detected; and if adrenaline or noradrenaline, or the temporary vasoconstriction they produced, did release any histamine it cannot have exceeded 0.2 μg. Such a result implies that adrenaline and noradrenaline are of the order of 10,000 times less active than Compound 48/80, in releasing histamine, and may be totally inactive.

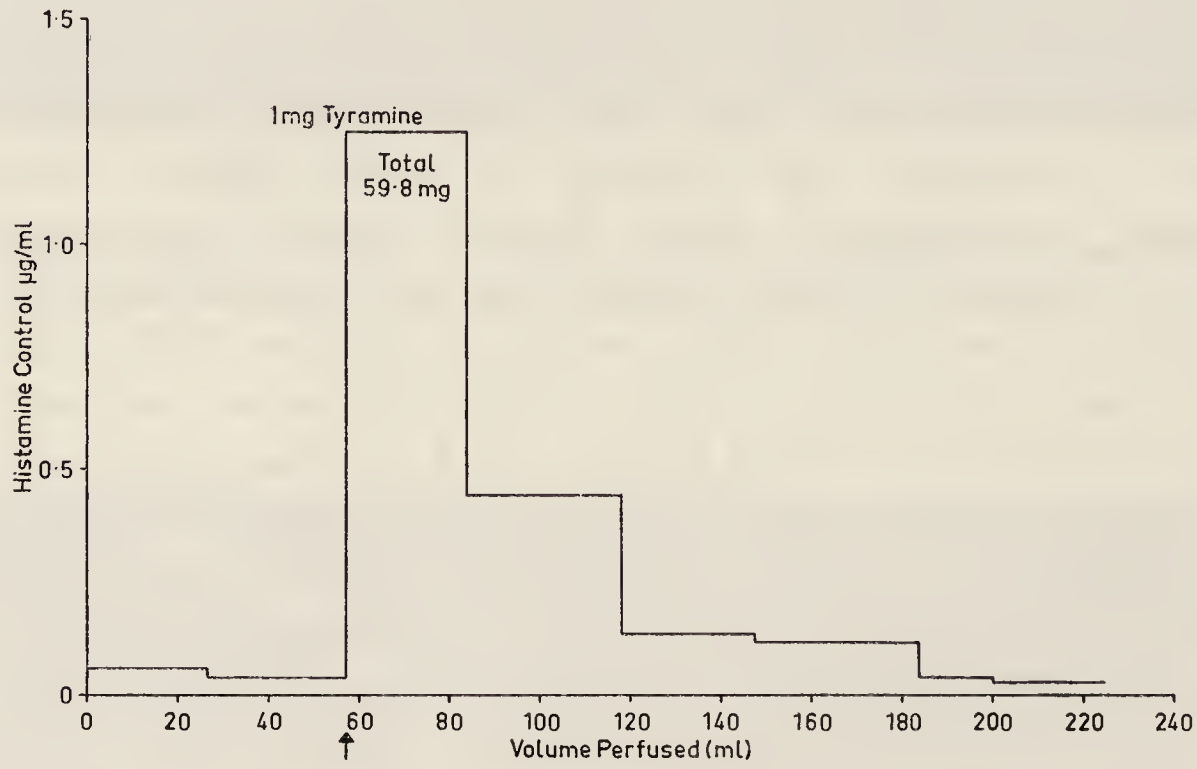

Fig. 10. Cat's isolated perfused skin. Output of histamine in response to 1 mg. tyramine.

A final set of experiments were made with certain other sympathomimetic amines, since the structure of Compound 48/80 can be regarded as that of a substituted polymerised tyramine. It was found that tyramine (fig. 10), β-phenylethylamine and amphetamine could release histamine from the cat's skin: 1 mg. tyramine mobilized 59.8 μg. histamine; 10 mg. β-phenylethylamine mobilized 121.7 μg. histamine; 10 mg. amphetamine, 12 μg. histamine. Methedrine (10 mg.) and propadrine (10 mg.), however, were nearly inactive (less than 1 μg. release), and ephedrine (10 mg.) appeared completely so. Intradermally, tyramine 0.1 % and amphetamine 1 % both produced wheals about 8 mm. in diameter and surrounding flares about 30 mm. diameter, but 0.1 % ephedrine was indistinguishable from saline.

Discussion

These experiments were undertaken initially to elucidate the action of *Heidenhain*'s first order lymphagogues. The finding that they release histamine, and that histamine-liberators also promote lymph flow, reduces the action of these lymphagogues to that of a single well-known substance, and renders it unnecessary to regard them as individual capillary poisons. Such a conclusion is fully concordant with *Starling*'s view that they increase the permeability of the capillaries and dilate the blood vessels. This mode of action also accounts for urticarial or other anaphylactoid reactions in the absence of previous sensitization, although no doubt sensitization can also occur. As histamine-liberators, they are interesting as providing further examples of such activity occurring in products of natural origin.

The lymphagogue action of adrenaline and noradrenaline, however, must have another explanation. The evidence against direct histamine release by these amines is primarily that no histamine could be detected in free form after their administration. But a negative result, particularly with substances whose own action may interfere with the assay of histamine, tends to leave some doubts. For this reason, the results with other sympathetic amines, although limited, are valuable, particularly the inactivity of ephedrine; for they show that as the structure of adrenaline is approached in the ethylamine chain, histamine releasing action dwindles. Thus, the addition of a β-hydroxyl group to methylamphetamine to give ephedrine, or to amphetamine to give propadrine, is accompanied by a reduction of activity; and methylation of the nitrogen also appears to reduce activity. The continued lymphagogue effect of repeated doses of adrenaline points to the same conclusion; for it is typical of histamine-liberators that their actions wane with repetition. The cause of the increased lymph flow with adrenaline or noradrenaline is susceptible of several alternative explanations. It may be that their cardiovascular effect is such as to produce a relatively increased capillary blood pressure in the region of lymph formation (the viscera principally) despite reduced blood flow elsewhere; this is not likely in view of the known visceral vasoconstriction to which they give rise. Alternatively they may themselves damage the capillary endothelium, either by causing too prolonged a local ischemia, or by a direct effect on permeability which is normally masked by the arteriolar constriction.

From the practical point of view histamine release by sympathomimetic amines might be relevant to their use in resuscitation or in nasal vasoconstriction. It has long been known that prolonged adrenaline or noradrenaline infusions, if terminated abruptly, may produce a state of peripheral circulatory failure not altogether unlike that due to histamine. But it seems clear that such shock can hardly be due to histamine release, and a cause for it must be sought in other vascular events. Release of histamine may be more significant with nasal decongestants, since relatively concentrated solutions of various amines are widely used. A common fault is after-congestion following the administration of particular sympathomimetic amines, a result which might well be in part a consequence of histamine release. It is interesting that ephedrine, which has a very good reputation for relief of nasal obstruction, is also ineffective in releasing histamine. An intradermal test of amines intended for nasal use might be a simple way of excluding an unwanted action of this sort.

In a somewhat less technical connection, one may wonder whether eating crustacea presents the gastronome with any risk of anaphylactoid response. It was found that 1 cc. of decoction, equivalent to about ¼ gm. of the original flesh, released 2–20 μg. histamine. For a nice lobster with 100–300 gm. of flesh, this gives a release of the order of 1–25 mgm. histamine. Of course, the gourmet does not suddenly perfuse the vessels of his own skin with extracts of the food he is eating. But the estimate seems high enough to suggest that anaphylactoid distress from a crustacean feast might not be beyond the reach of gluttony.

Summary

1. *Heidenhain*'s first order lymphagogues release histamine from cat's isolated perfused skin, and elicit a triple response in human skin if injected intradermally.

2. Representative histamine-liberators, propamidine, morphine, d-tubocurarine, and Compound 48/80, increase the flow of lymph from the thoracic duct in the cat.

It is concluded that the first order lymphagogues owe their action to release of histamine in the tissues.

3. Adrenaline and noradrenaline in large doses also cause considerable increase in lymph flow, but no evidence for histamine release by these amines could be obtained.

4. β-phenylethylamine and tyramine are effective histamine liberators; amphetamine is also active but less so; methylamphetamine and propadrine have just detectable action; ephedrine appears to be inactive.

5. The danger of eating lobster is discussed.

References

1. *Bainbridge, F. A.* and *Trevan, J. W.:* Some actions of adrenaline on the liver. J. Physiol. *51:* 460–468 (1917).
2. *Dale, H. H.* and *Richards, A. N.:* The depressor (vasodilator) action of adrenaline. J. Physiol. *63:* 201–210 (1927).
3. *Feldberg, W.* and *Paton, W. D. M.:* Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators. J. Physiol. *114:* 490–509 (1951).
4. *Heidenhain, R.:* Versuche und Fragen zur Lehre von der Lymphbildung. Pflügers Arch. ges. Physiol. *49:* 209–301 (1891).
5. *Mongar, J. L.* and *Whelan, R. F.:* Histamine release by adrenaline and d-tubocurarine in the human subject. J. Physiol. *120:* 146–154 (1953).
6. *Paton, W. D. M.:* Histamine release by compounds of simple chemical structure. Pharmacol. Rev. *9:* 269–328 (1957).
7. *Starling, E. H.:* On the mode of action of lymphagogues. J. Physiol. *17:* 30–47 (1894).

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